Supporting information

All reagents were obtained from Sigma-Aldrich and used as received unless noted. All solvents were dried over activated 4Å molecular sieves. LB broth was from Gibco. 1,3 diaminopropane was distilled onto molecular sieves under reduced pressure. Silica gel was from ICN (silitech 32-63 D 60 Å). HPLC purification was carried out using a Supelco C16 Discovery column (25cm x 4.6 mm, 5µm) using a gradient of 0.08% TFA, 1% acetonitrile in water to 0.08% TFA 85% acetonitrile in water, over a period of 30 min. The signals were detected by fluorescence (ex. = 365, em. = 450) or by absorbance at 254 or 290 nm. TLC plates were Merck Silica gel 60 F_{254} and were visualized using ceric ammonium molybdenate, ninhydrin or UV irradiation at 254 nm or 365 nm. Mass spectrometry was performed at the Cornell Mass Spectrometry facility using electrospray ionization in the positive ion mode.

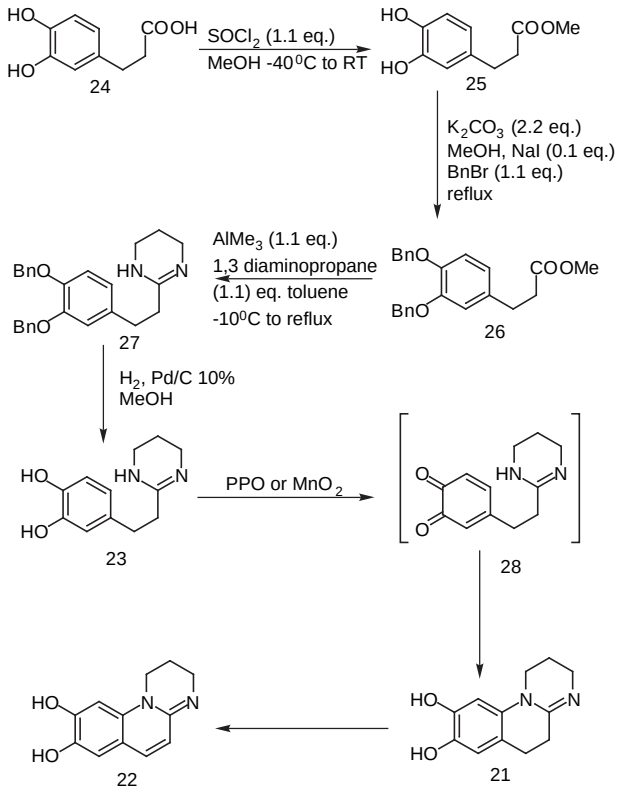

Figure 1S: Synthesis of **22.**

3-(3,4-dihydroxy-phenyl)propionic acid methyl ester (25):

3,5 dihydroxydihydrocinnamic acid (**24,** 3.0g, 16.5 mmol) was dissolved in 75 ml of MeOH and cooled to -40 ^{0}C in an acetonitrile-dry ice bath. Thionyl chloride (1.2 ml, 32.8 mmol) was added dropwise with stirring and the reaction mixture was allowed to stand at room temperature for 24 hours. Solvent removal under vacuum gave **25** as a clear green oil quantitaively. ^{1}H-NMR ($CDCl_3$, 400 MHz) 6.72 (d, J=8.0, 1H), 6.67 (s, 1H), 6.53 (d, J=8.0, 1H), 6.0 (br.s, 2H), 3.65 (s, 3H), 2.79 (t, J=8.0, 2H), 2.57 (t, J=7.6, 2H). ^{13}C-NMR ($CDCl_3$, 400 MHz) 175.99, 145.03, 143.47, 133.95, 121.37, 116.68, 116.61, 53.07, 37.03, 31.25. IR (neat, cm^{-1}) 3394 (br.), 3031, 2954, 1712 (ester), 1612, 1520.

3-(3,4-bis-benzyloxy-phenyl)-propionic acid methyl ester (26):

K_2CO_3 (4.72 g, 34.2 mmol, previously dried at 200 ^{0}C for 12 hours) and NaI (0.24 g, 1.6 mmol) were added to a solution of 3-(3,4-dihydroxy-phenyl)propionic acid methyl ester (**25,** 3.2g, 16.3 mmol) in 75 ml of dry methanol under argon. After stirring at RT for 10 min, benzyl bromide (3.9 ml, 32.8 mmol) was added dropwise, the reaction mixture was heated at reflux for 5 hours, poured into 100 ml of cold water, concentrated to half the original volume by rotary evaporation and extracted with ethyl acetate (3x50 ml). The extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$) and the solvent removed. Chromatography (Silica, column prepared in $CHCl_3$ and eluted with 98:2 $CHCl_3$:MeOH) gave **26** as a colorless oil which is moderately volatile under high vacuum (2.52g, 39%). TLC (90:10 $CHCl_3$:MeOH) Rf 0.85, ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.41 (m, 10H), 6.87 (d, J=8.4, 2H), 6.83 (s, 1H), 6.73 (d, J=8.0, 1H), 5.15 (s, 2H), 5.14 (s, 2H), 3.65 (s, 3H), 2.87 (t, J=8.0, 2H), 2.58 (t, J=8.0, 2H). IR (neat, cm^{-1}) 3533, 3448, 3062, 3032, 2947, 2870, 1959, 1874, 1735 (ester), 1589, 1512.

2-[2-(3,4-bis-benzyloxy-phenyl)-ethyl]-1,4,5,6-tetrahydro-pyrimidine (27):

1,3 diaminopropane (25 µl, 300 µmol) was added to a solution of $AlMe_3$ (160 µl of a 2M solution in toluene, 320 µmol) in 1.8 ml of dry toluene at –10^{0}C (NaCl-ice bath)

under argon.[1] After stirring for 15 minutes, a solution of 3-(3,4-bis-benzyloxy-phenyl)-propionic acid methyl ester (**26**, 100 mg., 269 μmol) in 1 ml toluene was added dropwise. The reaction mixture was heated at reflux for 4 hours, cooled to 0^0C, quenched with cold water (200 μl) and MeOH (2 ml) and cooled to -10^0C to further precipitate the solid. Filtration through celite followed by removal of the solvent gave **27** contaminated with the diamine. This was removed by storing the sample under vacuum (67 mTorr) for six days to give the product as a clear yellow wax like solid which decomposed on silica gel (105 mg, 98 %). ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.30 (m, 10H), 6.80 (s+d, 2H), 6.67 (d, J=8.0, 1H), 5.10 (s+s, 4H), 3.20 (t, J=6.0, 4H), 2.77 (t, J=8.4, 2H), 2.67 (t, J=7.2, 2H), 1.65 (quin., J=5.6, 2H). ^{13}C-NMR ($CDCl_3$, 400 MHz) 162.0, 149.9, 148.4, 138.4, 134.7,129.5, 128.8, 128.4, 122.4, 116.6, 116.2, 78.5, 72.4, 72.2, 53.8, 40.5, 36.6, 33.9, 20.0. IR (neat, cm^{-1}) 3163, 3032, 1931, 2785, 1651(amidine), 1512. MS (m/z, $M+H^+$) 401.

4-[2-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-ethyl]-benzene-1,2-diol (23):

Palladium on carbon (10%, 93 mg) was added to a solution of 2-[2-(3,4-bis-benzyloxy-phenyl)-ethyl]1,4,5,6-tetrahydro-pyrimidine (**27,** 86mg, 215 μmol) in 15 ml of degassed MeOH. After bubbling hydrogen through this solution for 1-1.5 hours and purging with argon for two minutes, the reaction mixture was filtered through a pad of celite (4 mm), the filtercake was washed with 10 ml of MeOH and the solvent was removed from the combined filtrate and washings. It is important that the filtration step is done under an inert atmosphere, otherwise **21** and **22** will form. Trituration of the resulting solid with $CHCl_3$ (1 ml) followed by drying under vacuum give the product as a colorless solid (41 mg, 87%). m.p. 63.1-64.9ºC. HPLC (290 nm) 4.0 min. ^{1}H-NMR (D_2O, 400 MHz) 6.07 (d, J=7.6, 1H), 6.60 (s, 1H), 6.49 (d, J=8.0, 1H), 3.10 (t, J=6.0, 4H), 2.67 (t, J=7.6, 2H), 2.45 (t, J=6.8, 2H), 1.66 (quin., J=6.0, 2H). ^{13}C-NMR (D_2O, 400 MHz) 163.8, 145.3, 143.9, 132.1, 121.6, 117.2, 117.1, 39.3, 35.4, 32.4, 18.3. MS (m/z , $M+H^+$) 221.

[1] Moormann, A. E.; Pitzele, B. S.; Jones, P. H.; Gullikson, G. W.; Albin, D.; Yu, S. S.; Bianchi, R. G.; S., E. L.; Rubin, B.; **1990**, J. Med. Chem. 33, 614-26.

MnO_2 oxidation[2] of 23 to 22:

Activated molecular sieves (approx. 2ml) and MnO_2 (21.7 mg, 250 μmol) were added to a suspension of 4-[2-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-ethyl]-benzene-1,2-diol (**23,** 11 mg, 50 μmol) in 5 ml of dry acetone under an argon atmosphere. The reaction mixture was heated at reflux with stirring for 3 hours and filtered through a 4 mm celite pad. The filter is washed, first with dry acetone (10 ml) then with a 50:50 mixture of methanol and water (20-40 ml) until all of the fluorescent material has been extracted. Half of the solvent was removed by rotary evaporation and the resulting highly fluorescent solution (366 nm) was lyophilized to give 9 mg of a green clear solid. The fluorescent product (and its acetylated and methylated derivatives) is not stable on Silica, Florisil, or Alumina and was purified using cation exchange chromatography[3] (Amberlite CG-50, 6 g, 20 cm x 10 mm column) using isocratic elution with 1 mM HCl (100 ml), followed by gradient elution with 1 mM (100 ml) to 100 mM HCl (100 ml), followed by isocratic elution with 100 mM HCl, (100ml) followed by gradient elution with 100 mM (100 ml) to 1M HCl (100 ml), followed by isocratic elution with 1 M HCl (100 ml). Compound **22** was detected by its fluorescence and was contained in a ~75 ml fraction eluting with the 100 mM to 1M HCl gradient. The blue fluorescent fractions were combined and lyophilized. ^{1}H-NMR (400 MHz, D_2O), 7.62 (d, J=8.4, 1H), 6.99 (s, 1H), 6.97 (s,1H), 6.50 (d, J=9.6, 1H), 4.05 (br.t., 2H), 3.32 (br.t.,2H), 2.05 (br.m., 2H). MS (m/z, $M+H^+$) 217, Daughter ion (MS MS, m/z, M^+) 217, 189, 160. HPLC (Ex = 365, Em = 450) 13.1 min., UV-vis (1:1 MeOH: H_2O, λ, pH=7.5) 390, 265, 233nm.

Polyphenol oxidase catalyzed oxidation of 23 to 22:

The procedure used was identical to that used for the polyphenol oxidase catalyzed oxidation of **20** to **21** and **22** described below.

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[2] Hirano, M.; Yakabe, S.; Chikamori, H.; Clark J. H.; Morimoto, T.; J. Chem. research (S), **1998**, 770-771.

[3] The column was prepared as described in Himmeldirk, K.; Sayer, G. S.; Spenser I. D.; *J. Am. Chem. Soc.,* **1998**, 120, 3581-3589.

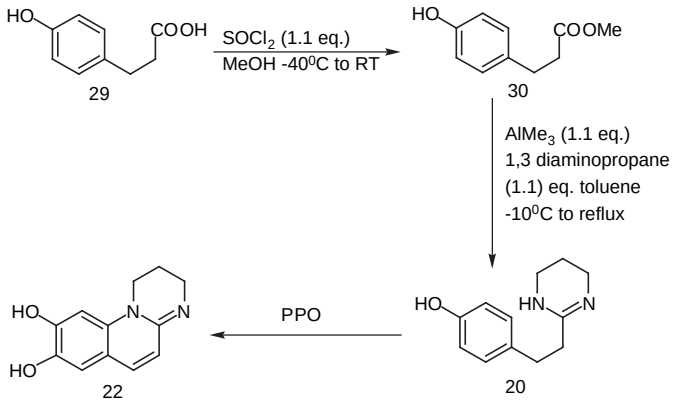

Figure 2: Synthesis of **22** from phenol **20**.

3-(4-hydroxy-phenyl)-propionic acid methyl ester (30):

Thionyl chloride (1.2 ml, 16.5 mmol) was added dropwise with stirring to a solution of **29** (2.75 g, 16.5 mmol) in 75 ml of MeOH at -40 ^{0}C (acetonitrile-dry ice bath). The reaction mixture was stirred at room temperature for 24 hours. Removal of the solvent and excess thionyl chloride under reduced pressure gave the product as a clear yellow liquid (2.88g, 96 %). ^{1}H-NMR ($CDCl_3$, 400 MHz), 7.01 (d, J=7.2, 2H), 6.73 (d, J=7.6, 2H), 5.21 (br. S., 1H), 3.65 (s, 3H), 2.85 (t, J=7.6, 2H), 2.59 (t, J=7.2, 2H). ^{13}C-NMR ($CDCl_3$, 400 MHz), 31.26, 37.35, 53.34, 116.69, 130.55, 133.04, 155.60, 175.85. IR (neat, cm^{-1}) 3396, 3022, 2952, 1712 (ester), 1614, 1518.

4-[2-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-ethyl]-phenol (20):

1,3 diaminopropane (100 µl, 1.2 mmol) was added to a solution of $AlMe_3$ (800 µl of a 2M solution in toluene, 1.6 mmol) in 1.8 ml of dry toluene at –10^{0}C (NaCl-ice bath) under argon. After stirring for 15 minutes, a solution of 3-(4-hydroxy-phenyl)-propionic acid methyl ester (**30**, 175 mg, 0.97mmol) in 4 ml toluene was added dropwise. The reaction mixture was heated at reflux for 4 hours, cooled to 0^{0}C, quenched with cold water (400 µl) and MeOH (4 ml) and cooled to -10^{0}C to further precipitate the solid. Filtration through celite followed by removal of the solvent gave **20** contaminated with the diamine. This was removed by storing the sample under vacuum (20 mTorr) for six days to give the

product as a a white crystalline solid (179mg, 89%). ^{1}H-NMR (D_2O, 400 MHz) 6.82 (d, J=7.6, 2H), 6.52 (d, J=7.6 2H), 3.02 (t, J=5.6, 4H), 2.62 (t, J=6.8, 2H), 2.39 (t, J=7.2, 2H), 1.58 (quin., J=6.0, 2H). ^{13}C-NMR (D_2O, 400 MHz) 18.30, 32.24, 35.58, 39.25, 118.25, 127.89, 130.66, 130.73, 163.89. MS (m/z, $M+H^+$) 205. IR (neat, cm^{-1}) 3400 (br.), 2949, 2837, 1655 (amidine), 1450, 1409, 1111, 1023.

Polyphenol oxidase catalyzed oxidation of 4-[2-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-ethyl]-phenol (20):

A solution of **20 (**3 mg) in 15ml of 40 mM KPi at pH 6.8 was incubated with polyphenol oxidase (40 μg) until the fluorescence reached its maximum value[4] (20-40 min.). The reaction mixture was then quenched with methanol and filtered through a membrane by centrifugation (5000 Da cutoff). The methanol was removed under reduced pressure and the water was removed by lyophilization. The fluorescent product was purified by cation exchange chromatography (Amberlite CG-50, 6 g, 20cm x 10mm, 1.6 ml/min.) using a gradient of 0.001M HCl (200 ml) to 0.2 M HCl (200 ml). The fluorescent fractions were lyophilized to give **22 (**200μg, 6%) as a green glass-like solid[5]. ^{1}H-NMR (400 MHz, D_2O), 7.62 (d, J=8.4, 1H), 6.99(s, 1H), 6.97 (s,1H), 6.50 (d, J=9.6, 1H), 4.05 (br.t., 2H), 3.32 (br.t.,2H), 2.05 (br.m., 2H). HPLC (ex = 365, em = 450) 13.1 min. Fluorescence em. (1:1 MeOH: H_2O, pH 7.5, ex. 390 ,λ_{max}) 445. UV-vis (1:1 MeOH: H_2O, λ) pH 9.5; 406 (ε = $1x10^5$), 235, 213, pH 7.5; 391, 265, 233, pH 3.5; 359, 308, 248, 220. MS ESI (m/z, $M+H^+$) 217. Daughter ion 217 (MS MS, m/z, $M+H^+$) 217, 189 (retro Diels-Alder), 160 (loss of nitrile). A second, light green fluorescent fraction was isolated upon solvent removal gave **21 (**400 μg, 12%) as a green solid. ^{1}H-NMR (400 MHz, D_2O), 6.64 (s, 1H), 6.60 (s,1H), 3.66 (br.t.,2H), 3.51 (br.t., 2H), 3.27 (br.m., 4H), 2.57 (s,4H), 2.01 (m,2H). UV-vis (1:1 MeOH:H_2O, pH 7.5, λ) 300, 440 (br.). MS ESI (m/z, $M+H^+$) 219. Daughter ion 219 (MS MS, m/z, M^+) 219, 191 (retro Diels-Alder), 158 (loss of nitrile). **21** re-incubated with polyphenol oxidase forms **22.**

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[4] Detected using Turner™ fluorimeter model 450 fitted with a excitation filter at 360 nm and an emission filter with a cutoff below 430 nm.

[5] A pronounced pH dependence of the absorbance of free pyoverdine is observed and ranges from 410 nm. at pH 9.6 to 370 nm at pH 3.0. At pH 3.0 a splitting is observed for pyoverdine and for **22**.

Polyphenol oxidase catalyzed oxidation of 21:

Polyphenol oxidase (40μg) was added to 400μg (2 μmol) of **21** in 4 ml of 40 mM triethanol amine (pH =7.5). After the fluorescence reached its maximum value, the reaction mixture was filtered through a 5000 kDa membrane, frozen, lyophilized. ESI-MS analysis demonstrated the formation of **22**.

Preparation of *Pseudomonas aeruginosa* cell free lysate:

Pseudomonas aeruginosa PA01 was grown on LB/agar plates at 37 ^{0}C for 12 hours and a single colony from this plate was used to inoculate 3 ml of LB medium. This starter culture was grown at 37 ^{0}C for 7 hours. Two flasks, each containing 1L of succinate based minimal medium[6] were inoculated with 1ml of this starter culture. $FeCl_3$ (100μL of a 0.4M solution in 0.1M HCl) was added to one of these flasks to give a final iron concentration of 40 μM. After incubation at 37 ^{0}C for 24 hours, the cultures were centrifuged (5000g) and the resulting cell pellets were washed with buffer (40 mM Kpi, pH 7.0, 2x20 ml), and pelleted by centrifugation. The washed cell pellets were resuspended in 5 volumes of buffer (40 mM Kpi, pH 7.0) and lysed by sonication. The cell lysates were centrifuged at 1000g, gel filtered (Bio-Rad**,** micro bio-spin 6 chromatography columns) to remove the small molecules and used immediately.

Protein concentration determination:

Protein concentration was determined using the Pierce Coomassie plus reagent with bovine serum albumin as the standard.

Activity assay:

Compound **20 (**0.5ml of a 1.0 mM stock solution in 40 mM KPi, pH 7.0) was incubated with 20μl (100-200 μg protein) of cell extract. After two hours, 0.5 ml of MeOH was added with thorough mixing to quench the reaction. 0.25 ml of this solution was added

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[6] One liter of medium contained 4.0 g KH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$ and 4.0 g succinic acid. The pH was adjusted to 7.0 with 2M KOH(aq.).

to 2.0 ml of 40 mM KPi pH 7.0 and the fluorescence recorded.[4] An identical protocol was followed for the other assays described in Table 1.

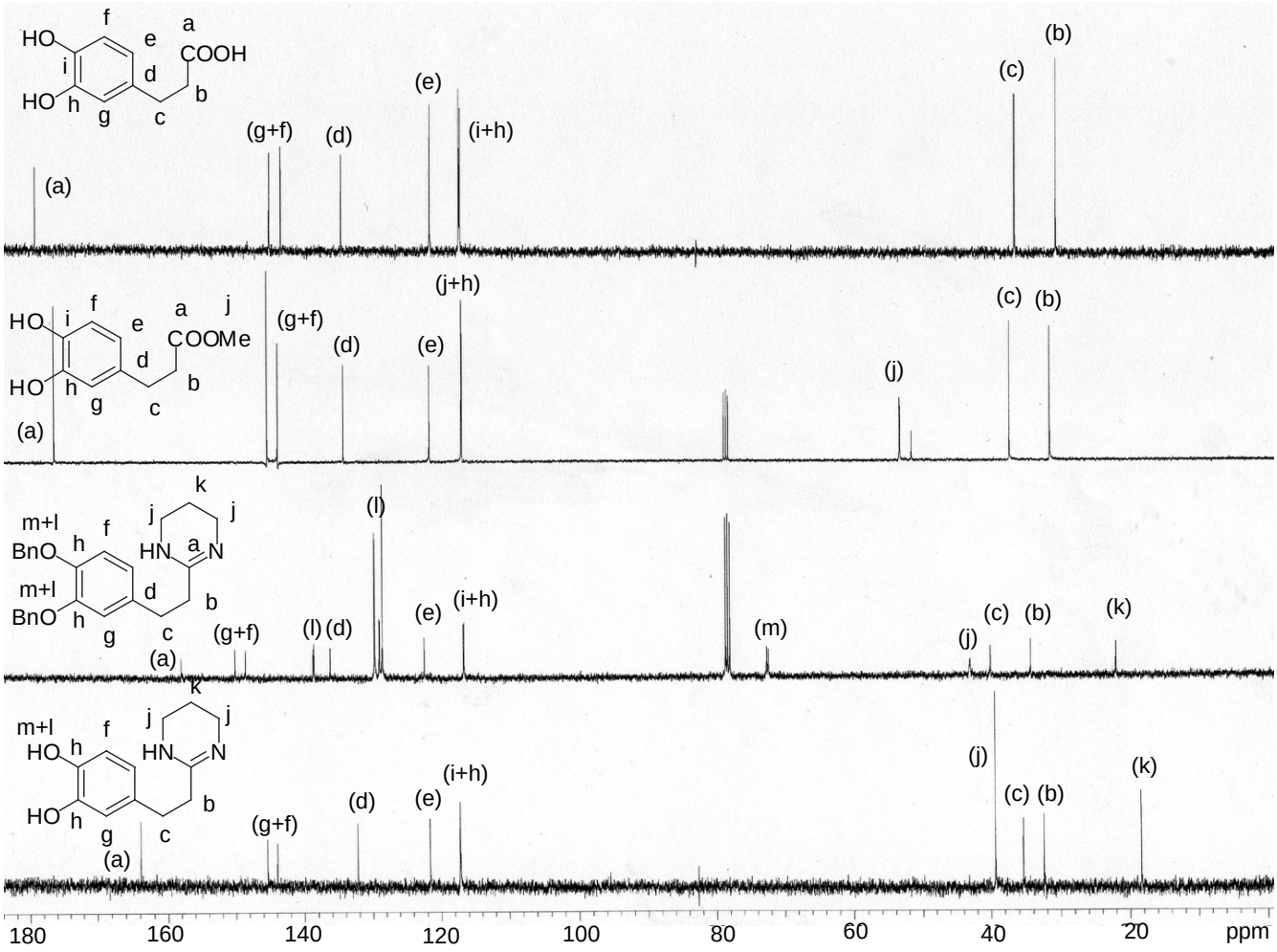

Figure 3S: ^{13}C-NMR Spectra of **24** (as obtained from Sigma-Aldrich), **25**, **27** and **23**.

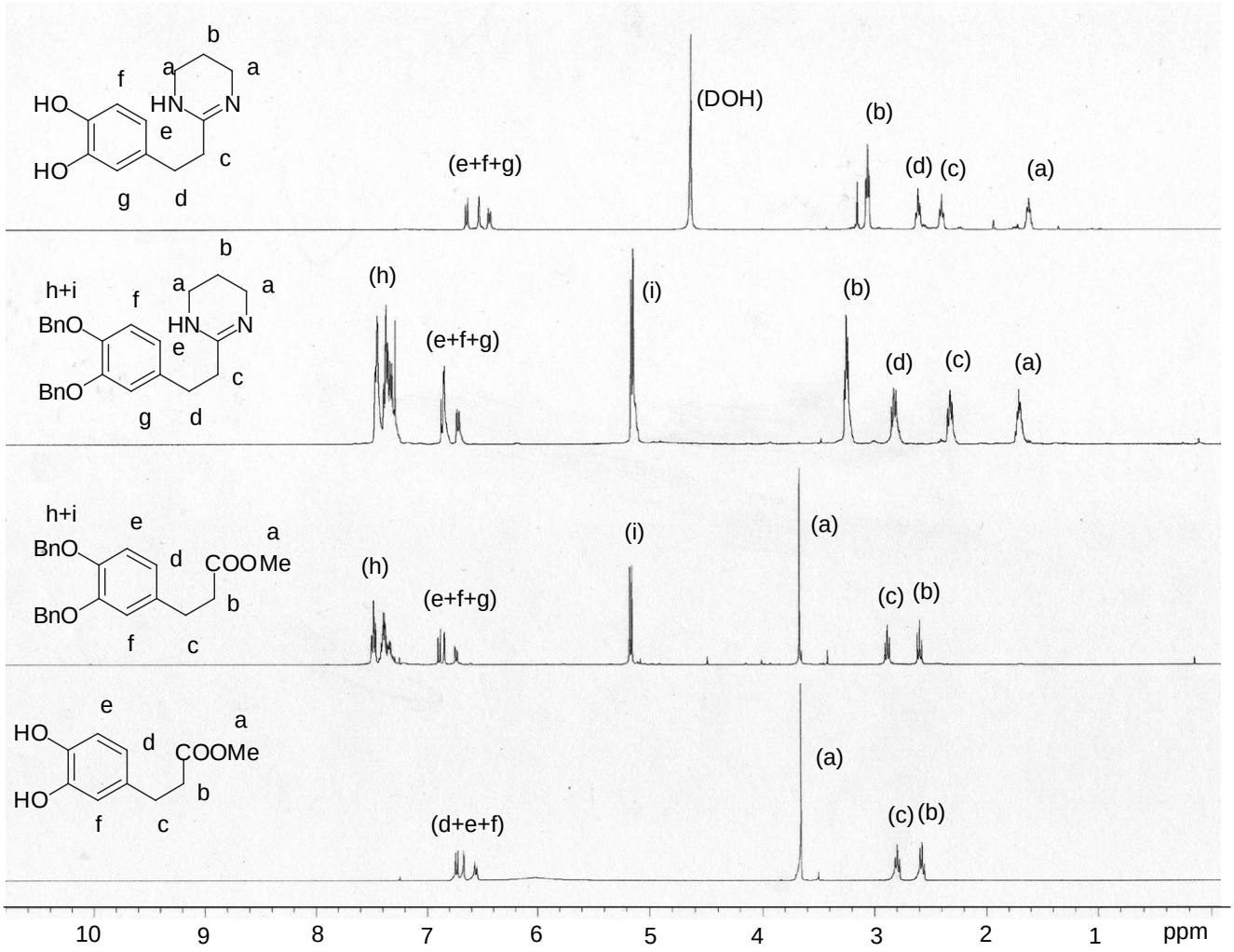

Figure 4S: ^{1}H-NMR Spectra of **25**, **26**, **27** and **23**.

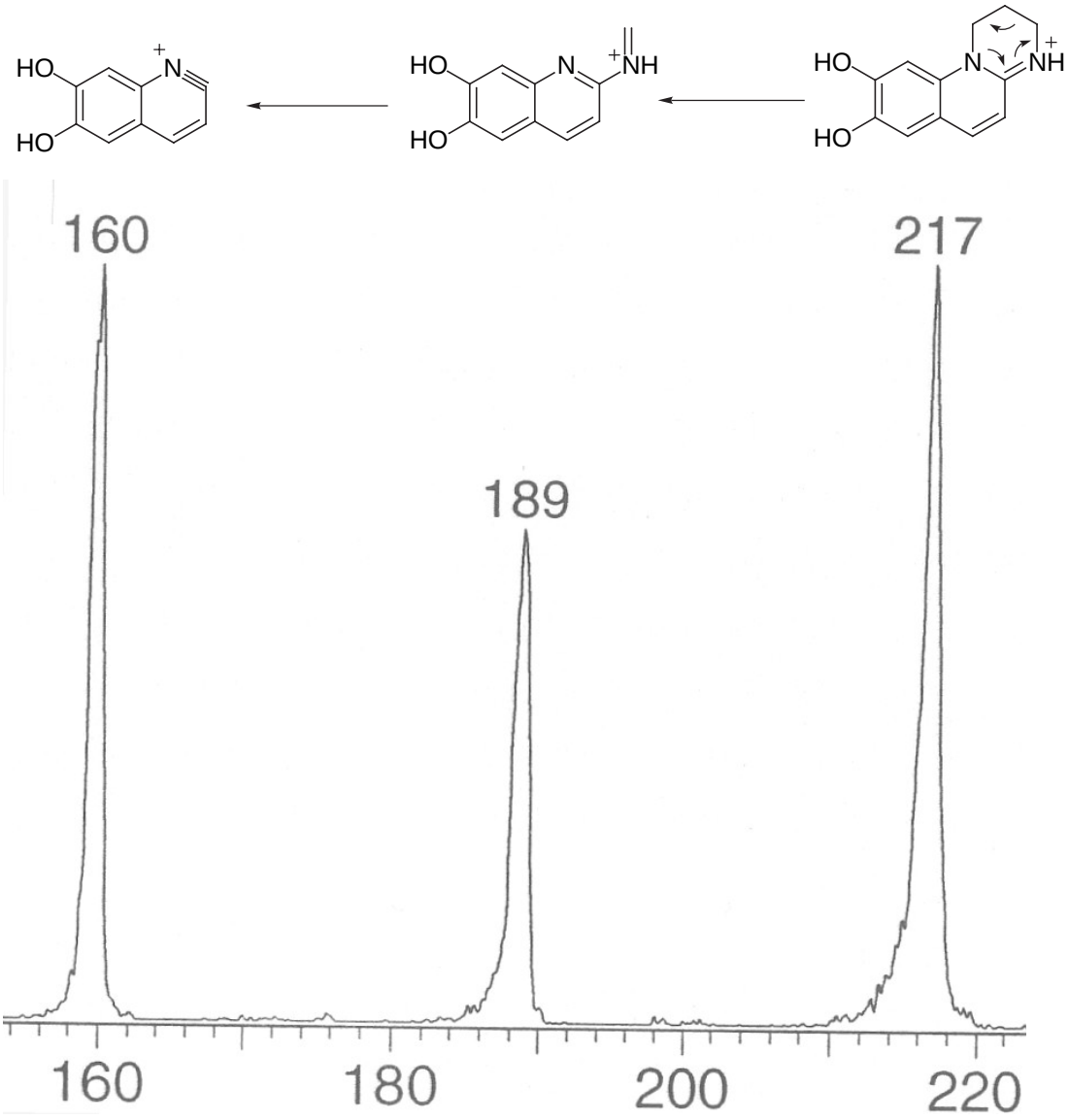

Figure 5S: Characteristic fragmentation pattern of **22** by ESI MS.